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MALATHION RESISTANT STRAINS OF *Aedes Aegypti* IN PUERTO RICO IN 1969¹

IRVING FOX AND ILEANA G. BAYONA

Department of Medical Zoology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico

Malathion is the favored insecticide against *Aedes aegypti* in Puerto Rico but its effectiveness is questionable. Following an epidemic of dengue in 1963, a large scale eradication program involved the spraying of hundreds of thousands of gallons of 2.5 percent solution over most of the Island at a cost of millions of dollars. The results were not impressive. In 1969, after five years of effort, *Aedes aegypti* was abundant everywhere, another epidemic of dengue broke out, and malathion continued to be applied in huge quantities.

Although earlier studies had indicated that *Aedes aegypti* in Puerto Rico was resistant to various chlorinated hydrocarbon and organophosphorus insecticides (Fox, 1960, 1961; Fox and Garcia-Mall, 1961; Fox *et al.*, 1961), including malathion but before standard tests were available of OP insecticides, malathion was chosen as the program's basic insecticide in 1964. Flynn *et al.* (1964) interpreted results from standard laboratory tests which had become available by this time as representing satisfactory susceptibility levels. By 1968, however, after several years' field experience, experts were not satisfied with the performance of malathion and searched for more efficient insecticides by means of field tests (Regnier

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et al., 1971); nevertheless malathion was the insecticide of choice in 1969 and continued in use thereafter.

The present experiments were undertaken to find out by standard laboratory tests and analyses whether any strains of *Aedes aegypti* in Puerto Rico exhibited signs of resistance to malathion during the control operations in 1969.

MATERIALS AND METHODS. From April to November, 1969, we obtained larvae from 27 Puerto Rican towns and colonized one collection from each town through several generations. To make the larval tests, we exposed for 24 hours about 20 fourth instar larvae in 250 ml World Health Organization solutions in open half-pint cardboard containers at concentrations of 0.05, 0.10, 0.25, 0.50, and 1.00 parts per million malathion, replicated the tests four times with appropriate controls, and counted moribund larvae as dead to calculate the percentage mortalities. We made adult tests using the standard test kit with malathion impregnated papers (0.4 percent, 0.8 percent, 1.6 percent, and 3.2 percent) supplied by W.H.O. Applying the method of Litchfield and Wilcoxon (1949), we computed the LC_{50} , LC_{90} , and LC_{95} values and their 95 percent confidence limits. The Rank Method of Wilcoxon (1949) indicated whether the strains differed significantly from one another in their susceptibility to malathion as shown by the percentage mortalities resulting from the tests.

RESULTS. Table 1 gives the relative

toxicity of malathion against the larvae of a single strain from the towns of Humacao, Barceloneta, Guánica, and Arecibo expressed in terms of LC_{50} , LC_{90} , and LC_{95} values, and Figure 1 gives the dosage-

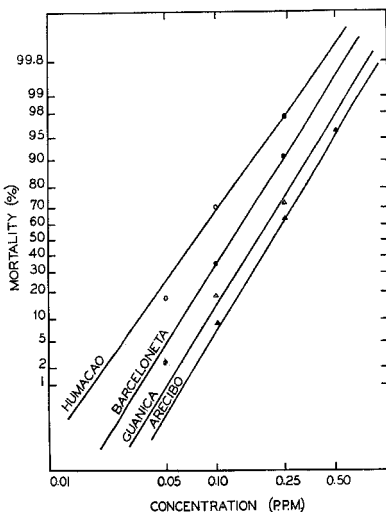


FIG. 1.—Dosage-mortality relationships of malathion and the larvae of *Aedes aegypti* from Puerto Rican towns.

mortality relationships. Wilcoxon's Rank Method indicated that results from these strains were significantly different from one another and that each of the remaining 23 strains was not significantly different from one or the other of these four, as shown in Table 3. Table 2 gives the LC_{50} , LC_{90} , and LC_{95} values in percent of malathion against adults of strains

TABLE 1.—Toxicity of malathion to *Aedes aegypti* fourth stage larvae after 24 hours' exposure as shown by LC_{50} , LC_{90} , and LC_{95} values in parts per million and confidence limits in parenthesis (average of four replicates of about 20 specimens each).

Town	LC_{50}	LC_{90}	LC_{95}
Humacao	0.07 (0.06-0.08)	0.16 (0.13-0.19)	0.20 (0.16-0.25)
Barceloneta	0.12 (0.10-0.14)	0.24 (0.19-0.30)	0.29 (0.23-0.37)
Guánica	0.17 (0.15-0.20)	0.34 (0.27-0.41)	0.40 (0.32-0.50)
Arecibo	0.21 (0.17-0.25)	0.40 (0.33-0.49)	0.48 (0.38-0.60)

TABLE 2.—Toxicity of malathion to *Aedes aegypti* adult females as shown by LC₅₀, LC₉₀, and LC₉₅ values in percent and confidence limits in parenthesis (average of four replicates of about 20 specimens each, one hour exposure followed by a 24-hour recovery period).

Town	LC ₅₀	LC ₉₀	LC ₉₅
Guayama	0.25 (0.21-0.29)	0.51 (0.39-0.66)	0.62 (0.45-0.86)
Aguadilla	0.40 (0.35-0.47)	0.76 (0.62-0.95)	0.92 (0.71-1.19)
Ponce	0.55 (0.49-0.62)	1.15 (0.97-1.37)	1.41 (1.15-1.73)
Fajardo	0.77 (0.66-0.90)	1.85 (1.46-2.35)	2.38 (1.79-3.17)
Arecibo	1.18 (1.00-1.39)	3.12 (2.33-4.18)	4.15 (2.94-5.85)

from Guayama, Aguadilla, Ponce, Fajardo and Arecibo. Figure 2 gives the dosage-mortality relationships of these five strains,

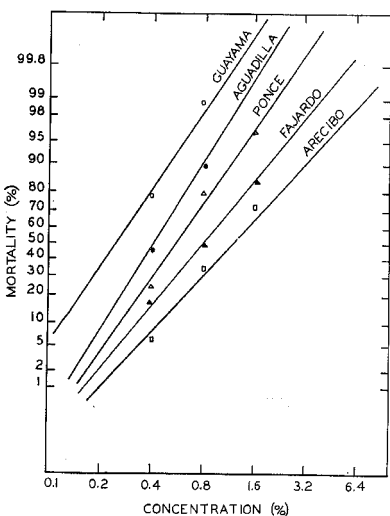


FIG. 2.—Dosage-mortality relationships of malathion and the adults of *Aedes aegypti* from Puerto Rican towns.

which differed significantly from each other according to Wilcoxon's Rank Method; and Table 3 shows that each of the remaining 22 strains did not differ significantly from one or the other of these five.

DISCUSSION. There are no clearcut standards concerning the resistance of

Aedes aegypti to malathion. Perhaps the data presented here, representing results of tests made on field strains during a period of malathion pressure, may help in setting such standards. Since the LC₅₀ values of larvae from strains throughout the world considered susceptible vary

TABLE 3.—The towns whose strains of *Aedes aegypti* did not differ significantly from the results of malathion laboratory tests shown in Figures 1 and 2.

Town	Not different from	
	Larva (Fig. 1)	Adult (Fig. 2)
Aguadilla	Barceloneta	
Arecibo		
Arroyo	Humacao	Aguadilla
Barceloneta		Guayama
Caguas	Barceloneta	
Carolina	Barceloneta	Guayama
Ceiba	Barceloneta	Ponce
Coamo	Barceloneta	Aguadilla
Corozal	Barceloneta	Guayama
Fajardo	Barceloneta	Caguas
Guánica		Guayama
Guayama	Barceloneta	
Guaynabo	Barceloneta	Guayama
Hatillo	Barceloneta	Guayama
Humacao		Guayama
Juncos	Guánica	Ponce
Lares	Humacao	Fajardo
Las Piedras	Barceloneta	Aguadilla
Loiza	Guánica	Aguadilla
Manati	Guánica	Ponce
Mayaguez	Barceloneta	Guayama
Patillas	Barceloneta	Guayama
Ponce	Guánica	
Quebradillas	Guánica	Fajardo
Rio Piedras	Barceloneta	Aguadilla
Santurce	Barceloneta	Aguadilla
Utua	Barceloneta	Ponce

from 0.06 to about 0.10 parts per million, the Puerto Rican strains represented by Humacao and Barceloneta (Table 3 and Figure 1) reacted normally but Guánica and Arecibo indicated resistance by a factor of about three. Resistance was more manifest in adults. Normally susceptible strains have LC_{50} values of about 0.30 percent; therefore, the strains represented by Guayama (Table 3 and Figure 2) were normal, but the others showed loss of susceptibility to various degrees. Arecibo in particular was resistant by a factor of about five. These data, the highest LC_{50} values for adults reported to the World Health Organization from anywhere in the World, together with control failure in the field after five years of operations, support our conclusion that segments of the population of *Aedes aegypti* in Puerto Rico in 1969 were resistant to malathion.

The imminent threat to Puerto Rico of epidemic dengue and other mosquito borne viruses makes it likely that large scale eradication programs may again be necessary in the future. Our data indicate the following ideas as regards such programs. First, although malathion may be necessary to control an epidemic in the initial, acute phase, it is unlikely to eradicate *Aedes aegypti* over the long run; second, continued extensive use of malathion will make the resistant strains more resistant and may lead to cross-resistance to other OP insecticides; and third, field and laboratory research to find more effective insecticides should be accomplished

using malathion resistant strains, particularly that from Arecibo.

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